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Experiences with contracts from National Institute of Health (NIH)

Ole Lund

Center for Biological Sequence Analysis

BioCentrum-DTU

Technical University of Denmark

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How we got involved

- Søren Buus post doc in USA in the 80'
- Collaboration with Alessandro Sette
- Science/Nature papers on binding of peptides to HLA
  - Which parts of microbes can be recognized by the immune system
  - The so called epitopes

Continued work separately

- Buus in DK
- Sette in USA
- My background:
- Engineer 1991 from Technical University of Denmark
- 1993-1998 Research assistant on Hvidovre Hospital
- 1998-2001 Structural Bioinformatics, Hørsholm
- 2001- Associate professor, Technical University of Denmark
  - Collaboration with Søren Buus

How we got involved II

- 9/11
- More focus on biodefence
- New research program (~$100 milioner)
  - Make database of all known epitopes
  - Discover new epitopes
  - Focus on A-C pathogens

The binding of an immunodominant 9-mer Vaccinia CTL epitope, HRP2 (KVDDTFYYV) to HLA-A*0201. Position 2 and 9 of the epitopes are buried deeply in the HLA class I molecule.

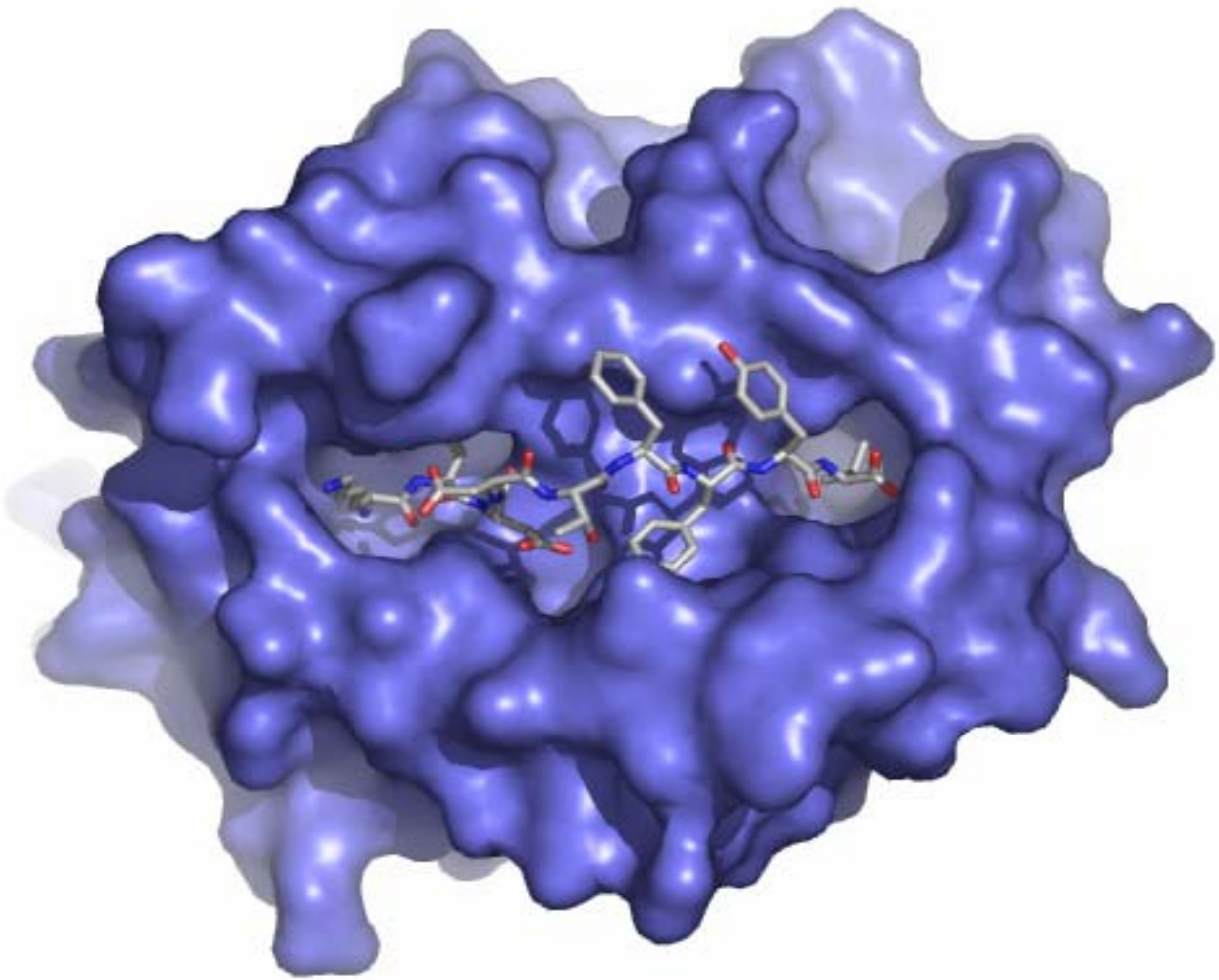

Figure by Anne Mølgaard, peptide (KVDDTFYYV) used as vaccine by Snyder et al. J Virol 78, 7052-60 (2004).

Request for proposals (RFP)

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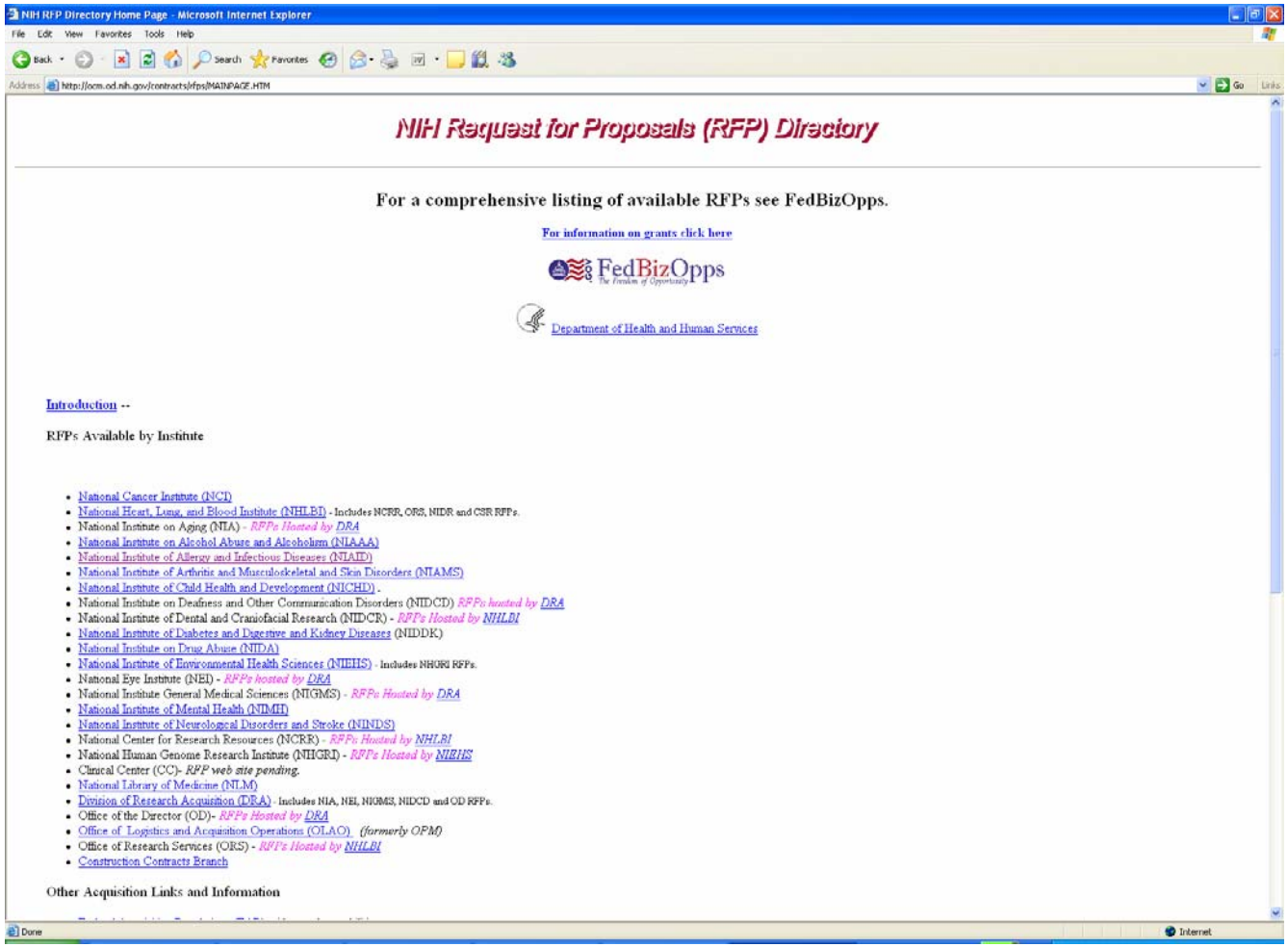
Correspond to Call for proposals i “EU speak”

- http://ocm.od.nih.gov/contracts/rfps/MAINPAGE.HTM



RFP I: Immune Epitope Database

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Project:

- Make database of all known epitopes
- Make it publicly available

Coordinator ”Principal investigator” (PI) on the application:

- Allesandro Sette, La Jolla Institute of Allergy and Immunology (LIAI)

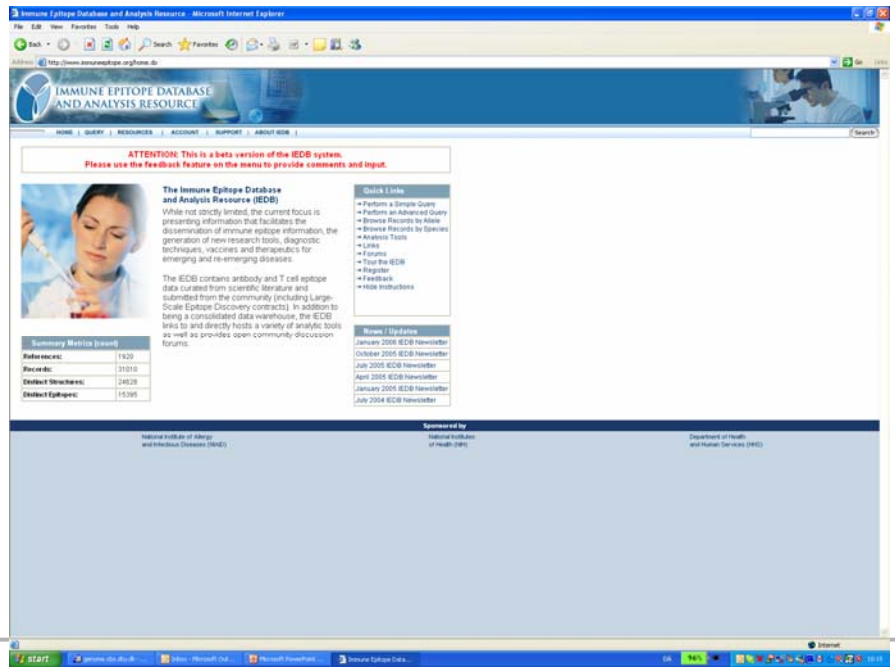
Subcontractors:

- Science Applications International Corporation (SAIC)
  - Largest employee-owned research and engineering company in the United States
  - ~43 000 employees
- San Diego Super Computing Center (SDSC)
- Panum (Søren Buus)
- DTU (Ole Lund)

Application of ~700 pages

Immune Epitope Database Contract

- Allesandro Sette wins the contract (~$25 milioner)
  - DK part ~1/2 employee for 5 years since December 2003
  - Send invoices each month
  - Phone conference every 2 weeks
  - Semi annual progress reports
  - http://www.immuneepitope.org/home.do



RFP II: Epitope Discovery I

Large Scale Antibody and T cell Discovery

Søren Buus PI

- DTU/Ole Lund is co-applicant
  - Hopelessly optimistic
- US co-applicant
- Søren Buus win one of several contracts
  - Goal: Find MHC binding peptides from A-C pathogens and develop better methods for prediction of MHC binding
- ~$3 million in the total of 5 years
- Started April 2004
- CBS part ~2,5 post doc + 15% administrator in
- Started April 2004

New Epitope Discovery RFP

Goal: To start more Epitope Discovery projects

- Identical to the earlier RFP

Ole Lund applies as PI

- Søren Buus, Mogens Claesson are co-applicants
- Focus for application:
  - Find epitopes in 15 microbes on the NIAID A-C list of pathogens that can be used for bioterror
  - Use MHC predictor from our earlier contract
  - http://www3.niaid.nih.gov/biodefense/bandc_priority.htm

Final Proposal Revision

Our application was “in the competitive range”
We were invited to write a Final proposal revision (FRP)
Corresponds to being invited to contract negotiations in EU

- Answer questions to
  - Technical proposal
  - Business proposal

Deadline is a few weeks after

- We made a 25 page answer to questions
- Last iterations

New Epitope discovery RFP

Ole Lund wins one contract (~$3,7 millions)

- Søren Buus, Mogens Claesson are subcontractors
- Started in October 2004
- CBS part ~2,5 post doc in 5 years

NIH Press Release

DRAFT	Media Contact: Paul Williams
FOR IMMEDIATE RELEASE	(301) 402-1663
Monday, Nov. 1, 2004	pw81y@nih.gov

NIAID Launches Program to Improve Medical Tools Against Emerging Infectious Diseases

The National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), has awarded 14 contracts totaling nearly $74 million to fund the Large-Scale Antibody and T Cell Epitope Discovery Program, an initiative aimed at quickly identifying the regions of select infectious agents that elicit immune reactions. The study of these regions, known as epitopes, promises to uncover targets for new and improved vaccines, therapies and diagnostic tools against potential bioterror agents as well as emerging/re-emerging infectious diseases such as West Nile virus and influenza. NIAID will make information on each newly identified epitope freely available to scientists through a searchable online database currently under development.

"Elucidating the basic mechanisms of immune function is a major piece of our biodefense research agenda," says Anthony S. Fauci, M.D., director of NIAID. "The information generated by this program will deepen our understanding of how components of the immune system defend against certain infectious agents, enabling researchers to design more effective medical countermeasures."

"Researchers have been conducting epitope discovery for many years, but generally on a small scale," says Daniel Rotrosen, M.D., director of NIAID's Division of Allergy, Immunology and Transplantation. "This initiative, however, will yield new knowledge about antigenic epitopes from a wide variety of microbes, including agents that might be used in a bioterrorist attack."

Obligations/Administration

- Help form a scientific advisory board
- Do the job specified in the statement of work
- Participate in yearly meetings in USA
  - All with a Epitope Discovery grant participates
- Administrator
  - Anne Christiansen

Reporting:

- In year 1 quarterly reporting
- In years 2-5 semi-annual reporting

Administration:

- Send invoice to NIH each month
- NIH are generally flexible but remember to ask for permission

Site visit/Audit

NIH can ask for a site visit
Project can be audited by a external company

NIH site-visit on Panum/CBS April 2006

- We invited NIH
- Learn a lot from their visit
- Obtain better understanding of what is/is not possible

Contract Title: Discovery of epitopes of NIAID category A-C pathogens using Bioinformatics and immunology

Contract No. HHSN266200400083C

Project goals

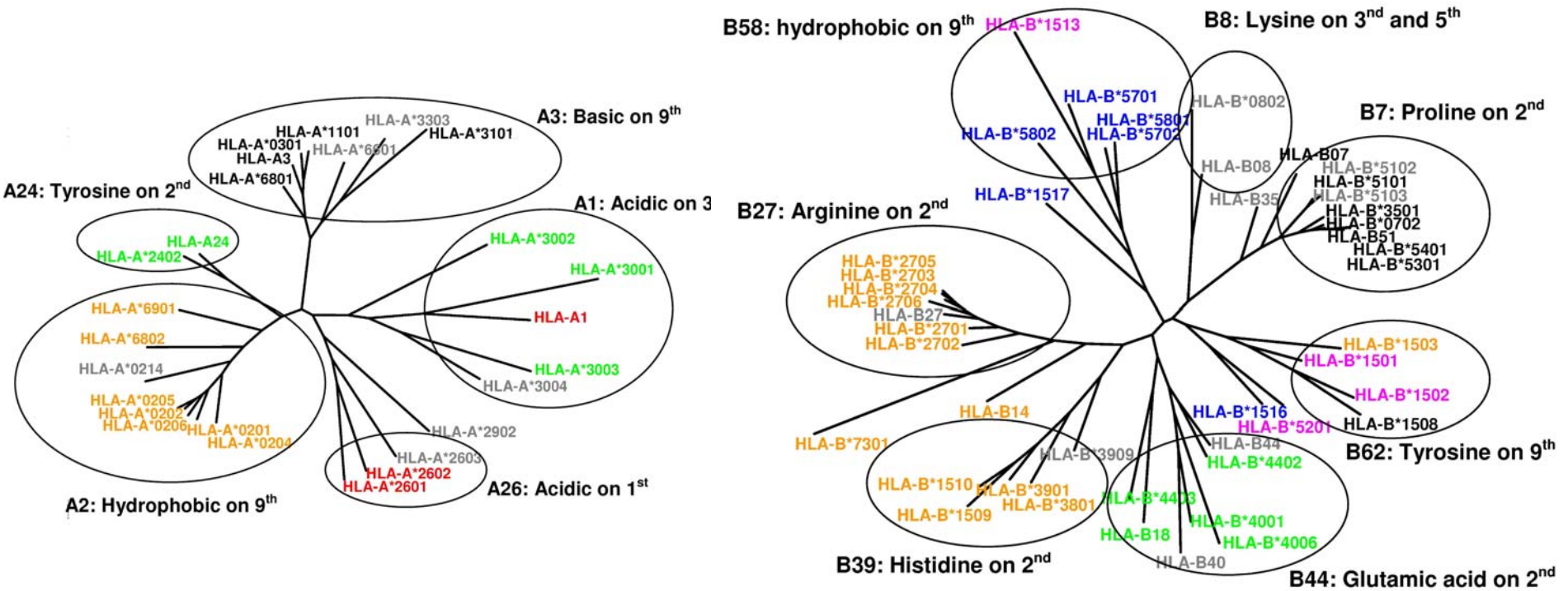
Develop improved methods to predict cytotoxic T cell (CTL) epitopes

Scan 15 different pathogens from the NIAID A-C list agents of bioterrorism

Test if cytotoxic T cells from preselected immune blood donors can react to the selected peptides for 3 selected pathogens: Influenza, Smallpox vaccine and tuberculosis vaccine (BCG)

Produce 5 new HLA molecules that are predicted to have a different specificity from the known supertypes

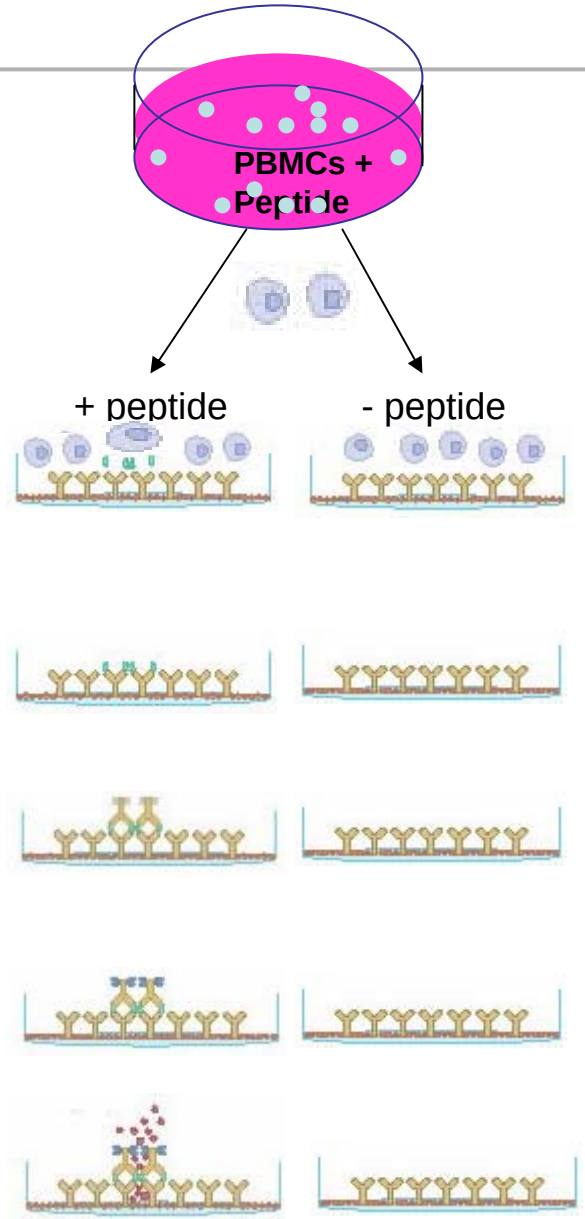
Clustering of HLA alleles



Clustering in: O Lund et al., Immunogenetics. 2004 55:797-810

Study is being updated in the Buus project using data from Buus and Sette

Flow Chart of ELISPOT Assay



Culture in vitro for 10 days + peptide

Incubating in anti IFN-γ pre-coated plate for 18-20 h

- Coating Ab:
  - –Human IFN-γ MAb (ENDOGEN, Pierce Biotechnology, Inc)

Washing off the cells

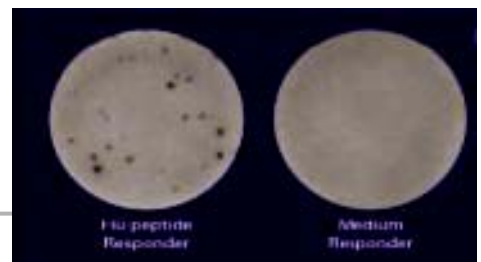
- Detection Ab:
  - –Human IFN-γ MAb, Biotin labeled
  - –(ENDOGEN, Pierce Biotechnology, Inc)

Adding Biotin-anti IFN-γ

Adding Streptavidin-HRP after washing the plate

Adding a substrate

Automatical counting



Selected pathogens

Pathogen	HLA binding	ELISPOT
Influenza	X	X
Variola major (smallpox) vaccine strain	X	X/VRC, NIH
Yersinia pestis	X	
Francisella tularensis (tularemia)	X	(X) A Sjostedt
LCM	X	
Lassa Fever	X	
Hantaan virus (Korean hemorrhagic fever virus)	X	
Rift Valley Fever	X	
Dengue	X	(X) T August
Ebola	X	
Marburg	X	
Multi-drug resistant TB (BCG vaccine)	X	X
Yellow fever	X	(X) T August
Typhus fever (Rickettsia prowazekii)	X	
West Nile Virus	X	(X) T August

Acknowledgements

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HLA binding

Claus Lundegaard
Data bases, HLA binding

Anne Mølgaard
MHC structure

Mette Voldby Larsen
Phd student - CTL prediction

Pernille Haste Andersen
PhD student – Structure

Sune Frankild
PhD student - Databases

Jens Pontoppidan
Linear B cell epitopes

Thomas Blicher
MHC structure

Sheila Tang
Pox/TB

Thomas Rask
Evolution

Nicolas Rapin/Ilka Hoff
Simulation of the immune system

Collaborators

IMMI, University of Copenhagen

Søren Buus	MHC binding
Mogens H Claesson	Elispot Assay

La Jolla Institute of Allergy and Infectious Diseases

Allesandro Sette	Epitope database
Bjoern Peters	

Leiden University Medical Center

Tom Ottenhoff	Tuberculosis
Michel Klein	
Fatima Kazi	

Ganymed

Ugur Sahin	Genetic library

University of Tubingen

Stefan Stevanovic	MHC ligands

INSERM

Peter van Endert	Tap binding

University of Mainz

Hansjörg Schild	Proteasome

Schafer-Nielsen

Claus Schafer-Nielsen	Peptide synthesis


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Anne Mølgaard
Morten Nielsen
Claus Lundegaard
Pernille Haste Andersen
Xiuxiu Ye
sgi
sgi altix 3000

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